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Environmental fate and effect assessment of thioridazine and its transformation products formed by photodegradation☆

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ABSTRACT

An experimental and *in silico* quantitative structure-activity relationship (QSAR) approach was applied to assess the environmental fate and effects of the antipsychotic drug Thioridazine (THI). The sunlight-driven attenuation of THI was simulated using a Xenon arc lamp. The photodegradation reached the complete primary elimination, whereas 97% of primary elimination and 11% of mineralization was achieved after 256 min of irradiation for the initial concentrations of 500 μg L^{-1} and 50 mg L^{-1}, respectively. A non-target approach for the identification and monitoring of transformation products (TPs) was adopted. The structure of the TPs was further elucidated using liquid chromatography–high resolution mass spectrometry (LC–HRMS). The proposed photodegradation pathway included sulfoxidation, hydroxylation, dehydroxylation, and S- and N-dealkylation, taking into account direct and indirect photolysis through a self-sensitizing process in the higher concentration studied. The biodegradability of THI and photolytic samples of THI was tested according to OECD 301D and 301F, showing that THI and the mixture of TPs were not readily biodegradable. Furthermore, THI was shown to be highly toxic to environmental bacteria using a modified luminescent bacteria test with *Vibrio fischeri*. This bacteriotoxic activity of THI was significantly reduced by phototransformation and individual concentration-response analysis confirmed a lowered bacterial toxicity for the sulfoxidation products Thioridazine-2-sulfoxide and Thioridazine-5-sulfoxide. Additionally, the applied QSAR models predicted statistical and rule-based positive alerts of mutagenic activities for carbazole derivative TPs (TP 355 and TP 339) formed through sulfoxide elimination, which would require further confirmatory *in vitro* validation tests.

1. Introduction

Pharmaceuticals are important micropollutants that have been subject of concern in the last decades (Kümmerer, 2001, 2009). They have been quantified up to μg L^{-1} in different environmental compartments such as hospital wastewaters and sewage treatment plants (STP), surface water, groundwater, drinking water (Lapworth et al., 2012; Rodil et al., 2012; Verlicchi et al., 2012) and even at sea water (Trautwein et al., 2014).

In the environment, pharmaceuticals are subject to biotic and abiotic reactions such as biodegradation, hydrolysis and photolysis that can result in the formation of transformation products (TPs) as a consequence of incomplete degradation (Fatta-Kassinos et al., 2011). The most common abiotic transformation process that pharmaceuticals are subjected to in surface waters is photolysis through sunlight (Lin et al., 2013; West and Rowland, 2012).

Very little is known about the fate and effects of TPs until now. They can be recalcitrant, persistent, and often they might show novel properties and activities, e.g. being even more toxic than the parent compounds (Fatta-Kassinos et al., 2011). Small changes in the structure of the parent compounds might lead to similar and

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non-target interactions on aquatic organisms, which are affected by the discharge of both parent compounds and TPs (Cwiertny et al., 2014). Therefore, knowledge on occurrence, fate and effects of TPs formed through photodegradation is of great importance to understand the potential risk for human health and the environment (Escher and Fenner, 2011). However, these TPs are often not accessible for experimental testing. This is why *in silico* models, such as quantitative structure-activity relationships (QSAR), have been used to support the environmental assessment of TPs (Mahmoud et al., 2014; Rastogi et al., 2014a, 2014b, 2015).

Psychiatric drugs, including phenothiazine pharmaceuticals, are worldwide prescribed drugs, being extensively used over the past years (Trautwein and Kümmerer, 2012b; Nałecz-Jawecki et al., 2008), and little attention has been given to their environmental fate in comparison to other pharmaceuticals micropollutants. One of the most commonly used phenothiazine pharmaceutical is Thioridazine (THI) achieving a total prescription amount of 153 kg in the year 2013 in Germany (Schwabe and Paffrath, 2014). In total, approximately 30% of orally administered THI is excreted in the urine and 50% of the original dose is excreted in the faeces (Eiduson and Geller, 1963). However, before excretion THI is extensively metabolized in the liver and of a daily dose of THI only 2.5%–7% is excreted as Thioridazine and conjugates, being 0.5% excreted as the parent compound, whereas 0.5% is excreted as Mesoridazine (THI-2-SO) and approximately 1% is excreted as Thioridazine-5-sulfoxide (THI-5-SO) via urine within 24 h (Baselt and Cravey, 1995). Thanacoody (2011) have pointed out that 4% of the original dose appears unchanged in urine. Recent studies have suggested THI as an affordable antimicrobial agent for the treatment of intracellular infections caused by multiresistant strains of *Mycobacterium tuberculosis* (pathogen of tuberculosis) and *Plasmodium falciparum* (pathogen of *Malaria tropica*) (Kristiansen et al., 2015; Thanacoody, 2007, 2011; Weismana et al., 2006). Further THI is a promising 'lead' compound used as antibiotic from the 'non-antibiotic' class and as a 'helper' compound combined with classical antibiotics for the treatment of multidrug-resistant Gram-negative infections (Martins et al., 2008; Worthington and Melander, 2013), and was even discussed as a promising drug for anti-cancer therapy (Nagel et al., 2012). Therefore, consumption and consequently release to the environment of THI and related compounds is expected to increase, especially in resource-poor countries plagued by endemic infectious diseases. At the same time, these countries are often lacking effluent treatment and are exposed to high sunlight intensity, which is why photodegradation in surface water is an important factor in the environmental risk assessment of THI.

Photodegradation of THI by VIS and UVA light has been investigated in the past mainly with regard to phototoxicity *in vitro* assay towards various biological substrates (Elisei et al., 2007; Miolo et al., 2006), and to the bioindicators *Spirostomum ambiguum* (Spirotox) and anostracan crustacean *Thamnocephalus platyurus* (Nałecz-Jawecki et al., 2008). The photochemical instability of phenothiazine pharmaceuticals can lead to phototoxic and photoallergic reactions in the human body (Nałecz-Jawecki et al., 2008). Besides, Nałecz-Jawecki et al. (2008) demonstrated that the protozoan *Spirostomum ambiguum* was very sensitive not only to the parent drugs but also to photodegraded solutions of THI and chlorpromazine. It is also known that psychiatric drugs such as carbamazepine can modulate behavior of aquatic organisms at concentrations in the range of 200–2000 ng L^{-1} and alter freshwater community structure and ecosystem dynamics (Jarvis et al., 2014a, 2014b). Together with the intrinsic antimicrobial activity, this suggests THI and TPs of THI as compounds of relevant environmental concern. However, there is only little information available on the environmental fate and effects of THI and its transformation products formed via photodegradation.

The aim of this study was to assess the environmental fate and effects of THI and its TPs formed after simulated sunlight irradiation. For that, the TPs were elucidated by means of ultra-high performance liquid chromatography–high resolution mass spectrometry (UHPLC–HRMS) using an Orbitrap mass spectrometer. The ready biodegradability of photodegraded samples was tested according to the OECD guidelines 301D and 301F. The impact of phototransformation on bacterial cytotoxicity was investigated using a modified luminescent bacteria test towards *Vibrio fischeri.* Besides, *in silico* QSAR tools were implemented for the initial assessment of mutagenicity of THI and its proposed TPs.

2. Material and methods

2.1. Chemicals

Thioridazine hydrochloride (≥99%, CAS No. 130-61-0), 3,5-Dichlorophenol (97%, CAS No. 591-35-5) and Chloramphenicol (98%, CAS No. 56-75-7) were purchased from Sigma-Aldrich (Deisenhofen, Germany). Thioridazine-2-Sulfoxide (CAS No. 32672-69-8) and Thioridazine-5-Sulfoxide (98%, CAS No.7776-05-8) were acquired from Santa Cruz Biotechnology (Dallas, Texas, USA). Organic solvents were of LC-MS grade and provided by VWR (Darmstadt, Germany). Aqueous solutions were prepared in ultrapure water (Q1:16.6 MΩ·cm and Q2:18.2 MΩ·cm, Ultra Clear UV TM, Barsbüttel, Germany). All other chemicals were of recognized analytical grade and used as received.

2.2. Photodegradation through simulated sunlight irradiation

The photodegradation experiments were carried out in a 1000 mL cylindrical immersion-type batch reactor with ilmasil quartz immersion tube using 800 mL of synthetic solutions of THI diluted in ultrapure water. The sunlight irradiation was simulated by means of an UV/VIS xenon lamp (TXE 150 W, UV Consulting Peschl, Mainz, Germany). The irradiance of the Xe lamp in the range 200–850 nm was measured with Black Comet UV–VIS spectroradiometer model C (StellarNet Inc., Florida, USA) showing the follow irradiance: 200–280 nm: 1.01 W m^{-2}; 280–315 nm: 3.29 W m^{-2}; 315–380 nm: 12.91 W m^{-2} and 380–850 nm: 243.16 W m^{-2}. The spectrum of the lamp and the molar extinction coefficient of THI are depicted in Text S1 (Supplementary material).

Initial concentrations of 500 μg L^{-1} and 50 mg L^{-1} of thioridazine hydrochloride (THI·HCl) were chosen in order to allow reliable experimental evaluations of toxicity, ready biodegradability and in order to produce TPs in a sufficient amount to allow their initial characterization and further assessment in a 'worst case' scenario. The experiments were carried out at pH 6.5 and no adjustments in pH of the solution were carried out during and after the experiments. A dark control in the same conditions as for photolysis was carried out by using an initial concentration of 500 μg L^{-1} of THI·HCl. The temperature was held at 20 (±2) °C with a circulating cooler (WKL230, LAUDA, Berlin, Germany).

2.3. Kinetic modeling of photodegradation

In addition to monitoring the primary elimination of the parent compound, the degree of mineralization is an important parameter and can help to establish an initial benchmark, being indirectly related to the presence of TPs and, consequently, to determine further biodegradation and toxicity studies. Thus, the non-purgeable organic carbon (NPOC) was monitored. According to Legrini et al. (1993), in general NPOC reductions follow an apparent zero order kinetic through direct photolysis. The experimental data of the NPOC removal were fitted in relation to a zero-order kinetic